CLAIMS

We claim:

1. A method for selecting proteins for displayability on a yeast cell surface, comprising:
transforming yeast cells with a vector which expresses a protein to be tested fused to a yeast cell wall protein, wherein mutagenesis is used to generate a variegated population of mutants of the protein to be tested;
contacting said yeast cells with an antibody or an antibody fragment which binds to proteins that are displayed on the yeast cell surface and does not bind to proteins that are not displayed on the yeast cell surface;
isolating said yeast cells with which said antibody or antibody fragment is bound, wherein the presence of said antibody or antibody fragment bound to a protein to be tested indicates said protein to be tested is displayable on the yeast cell surface.

2. A method of selecting T cell receptors displayed on a yeast cell surface with improved binding properties to desired labels comprising:
transforming yeast cells with a vector which expresses a T cell receptor fused to a yeast cell wall protein, wherein mutagenesis is used to generate a variegated population of mutants of the T cell receptor;
contacting said yeast cells with a label, wherein said label binds to T cell receptors that are displayed on the yeast cell surface and does not bind to T cell receptors that are not displayed on the yeast cell surface;
quantitating said label, wherein a high level of said label indicates the T cell receptor is displayed on a yeast cell surface and has improved binding properties to said label.

3. The method of claim 2, wherein said protein to be tested is a single chain T cell receptor and said label is a MHC-peptide complex or a superantigen.

4. A method of isolating proteins that bind to a region-specific label comprising:

transforming yeast cells with a vector which expresses a protein to be tested fused to a yeast cell wall protein, wherein mutagenesis is used to generate a variegated population of mutants of the protein to be tested;
contacting said yeast cells with a region-specific label which binds to a specific region of the protein to be tested;
isolating said yeast cells with which said region-specific label is bound.

5. A method of displaying more than one different polypeptide on the surface of yeast cells, comprising: transforming yeast cells with vectors which express proteins to be displayed fused to yeast cell wall proteins, wherein said polypeptides are one or more selected from the group consisting of those polypeptides of an organism which are displayable, and wherein said organism is a mammal.

6. A library comprising a plurality of different polypeptides displayed on yeast cells.

7. The library of claim 6, made by the method of displaying more than one different polypeptide on the surface of yeast cells, comprising:
transforming yeast cells with vectors which express proteins to be displayed fused to yeast cell wall proteins, wherein said proteins have an amino acid sequence consisting of a ligand binding polypeptide sequence joined at its N-terminus to the C-terminus of an agglutinin subunit Aga2p sequence, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

8. The library of claim 6, wherein said polypeptides are one or more selected from the group consisting of those polypeptides of an organism which are displayable.

9. The library of claim 8, wherein said organism is a mammal.

10. A fusion protein displayed on a yeast cell surface, the amino acid sequence of said fusion protein consisting of a polypeptide sequence joined at its N-terminus to the C-terminus of

an agglutinin subunit Aga2p sequence, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

11. The fusion protein of claim 10 wherein said amino acid sequence further includes a first epitope tag sequence between said Aga2p and polypeptide sequence.

12. The fusion protein of claim 11 wherein said amino acid sequence further includes a second epitope tag sequence joined to the C-terminus of said polypeptide sequence.

13. The fusion protein of claim 12 wherein a label is bound to said second epitope tag.

14. The fusion protein of claim 10 wherein a label is bound to said polypeptide.

15. The fusion protein of claim 10 wherein said polypeptide is a ligand binding polypeptide.

16. The fusion protein of claim 15 wherein said ligand binding polypeptide is chosen from the group consisting of functional antibodies and cell surface receptors and fragments thereof.

17. The fusion protein of claim 16 wherein said polypeptide is an anti-T cell receptor or a fragment thereof.

18. The fusion protein of claim 16 wherein said antibody or fragment thereof is an anti-T cell fragment chosen from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24.

19. The fusion protein of claim 16 wherein said polypeptide is a T cell receptor or fragment thereof.

20. The fusion protein of claim 19 wherein said T cell receptor or fragment thereof has been mutagenized.

21. The fusion protein of claim 20 wherein said mutant T cell receptor or fragment thereof is SEQ ID NO:24.

22. A yeast cell displaying a fusion protein having an amino acid sequence consisting of a ligand binding polypeptide sequence joined at its N-terminus to the C-terminus of an agglutinin subunit Aga2p sequence, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

23. The yeast cell of claim 22 wherein said fused polypeptide is capable of binding a specific ligand.

24. The yeast cell of claim 22 wherein said polypeptide sequence is a mutant of a related wild-type polypeptide.

25. The yeast cell of claim 22 wherein said yeast strain is of a genus selected from the group consisting of Saccharomyces, Pichia, Hansenula, Schizosaccharomyces, Kluyveromyces, Yarrowia, and Candida.

26. In a polypeptide displaying yeast cell, the improvement comprising:
a polypeptide fused to a yeast cell wall protein wherein said fused polypeptide is capable of binding a specific ligand; and
means for measuring the avidity and specificity of the fused polypeptide binding to said specific ligand.

27. In a yeast cell displaying a fusion protein, the improvement comprising:
said fusion protein containing a mutant ligand binding protein joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the mutant ligand binding protein being joined at its C-terminus to a second epitope tag, said Aga2p

being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

28. In a yeast cell displaying a fusion protein, the improvement comprising:
said fusion protein containing a mutated T cell binding protein joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the T cell binding protein sequence being joined at its C-terminus to a second epitope tag, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

29. In a yeast cell displaying a fusion protein, the improvement comprising:
said fusion protein containing a mutated antibody or fragment thereof capable of antigen-specific binding joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the antibody or fragment being joined at its C-terminus to a second epitope tag, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

30. A method of producing a yeast cell displayed variant ligand binding protein with enhanced binding properties relative to a wild-type of said ligand binding protein, the method comprising:
isolating a gene encoding said wild-type binding protein;
creating a library of mutated proteins by randomly mutating said wild-type protein;
incorporating each said mutated protein into respective expression cassettes, each having the structure

5'-GAL 1-10 -- a Aga2p -- mutated polypeptide;

incorporating each said expression cassette into a respective vector;

transforming yeast cells with said cassette-containing vectors to yield a multiplicity of transformed yeast cells;
expressing said cassettes in said transformed yeast cells, whereby said ligand binding protein is displayed on the surface of each said yeast cell, said displayed ligand binding protein containing one of said mutated binding;
labeling the displayed proteins on said yeast cells by binding a specific label to said displayed proteins;
employing flow cytometry to sort said yeast cells according to their labeling characteristics;
determining the surface expression level of said ligand binding protein in said sorted cells;
determining the ligand binding characteristics of said ligand binding protein on the surface of said sorted cells whereby at least one preferred yeast cell expressing an abundance of ligand binding protein which exhibits enhanced ligand binding characteristics is identified; and
cloning said at least one preferred yeast cell.

31. The method of claim 30 wherein said protein is joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the protein sequence being joined at its C-terminus to a second epitope tag, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface.

32. A method of producing a variant T cell binding protein with enhanced T-cell binding properties relative to a wild-type of said T cell binding protein, the method comprising:
isolating a gene encoding said wild-type T cell binding protein;
creating a library of mutated proteins by randomly mutating said wild-type protein;
incorporating each said mutated protein into respective expression cassettes, each having the structure

5'-GAL 1-10 -- a Aga2p --HA-- mutated polypeptide --c-myc-3';

incorporating each said expression cassette into a respective vector;
transforming yeast cells with said cassette-containing vectors to yield a multiplicity of transformed yeast cells;
expressing said cassettes in said transformed yeast cells, whereby a unique fusion protein is displayed on the surface of each said yeast cell, said fusion protein containing one of said mutated T cell binding proteins joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the T cell binding protein sequence being joined at its C-terminus to a second epitope tag, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface;
labeling the fusion proteins on said yeast cells by binding cytometrically distinguishable labels to said c-myc and to said T cell binding protein;
employing flow cytometry to sort said yeast cells according to their labeling characteristics;
determining the surface expression level of T cell binding protein in said sorted cells; and
determining the ligand binding characteristics of said T cell binding protein on the surface of said sorted cells whereby at least one preferred yeast cell expressing an abundance of fusion protein which exhibits enhanced T cell binding characteristics is identified;
cloning said at least on preferred yeast cell; and
reducing said disulfide bonds whereby said fusion protein is released from said yeast cells.

33. A variant Tcell product of the method of claim 32.

34. A process of developing a mutant polypeptide exhibiting more favorable binding of a predetermined ligand relative to the binding characteristics of a wild-type of said polypeptide for said ligand, the process comprising:
randomly mutating a predetermined wild-type polypeptide to yield a population of mutated polypeptides;

creating a library of yeast cells, each of which displays on its surface at least one copy of a fusion protein containing one of said mutated polypeptides, the amino acid sequence of said fusion protein consisting of said mutated polypeptide sequence joined at its N-terminus to the C-terminus of an agglutinin subunit Aga2p sequence, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface, a first epitope tag sequence between said Aga2p and ligand binding polypeptide sequences, and a second epitope tag sequence joined to the C-terminus of said ligand binding polypeptide sequence, wherein a label is bound to at least one of said second epitope tag and said mutant polypeptide;

sorting said yeast cells by flow cytometry;

cloning cells expressing a desired mutant polypeptide;

rescuing and sequencing the DNA sequence coding for said desired mutant polypeptide;

amplifying and expressing said DNA sequence; and

harvesting the desired mutant polypeptide.

35. The DNA intermediate product of the process of claim 34 said gene coding for the desired mutant polypeptide product of said process.

36. In a process for developing a protein with enhanced binding characteristics against a predetermined ligand relative to the binding characteristics of a wild-type of said protein for said ligand, in which the process includes mutating a gene encoding a wild-type of said protein, displaying on a yeast cell surface a mutant protein encoded by said mutant gene, contacting said ligand with said displayed mutant protein, and determining the extent of binding of ligand by said displayed mutant protein, the improvement comprising:

displaying on said yeast cell surface a fusion protein consisting of a mutant polypeptide sequence joined at its N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to the C-terminus of an agglutinin subunit Aga2p sequence, the mutant polypeptide sequence being joined at its C-terminus to a second epitope tag, said Aga2p being joined by two disulfide bonds to an agglutinin subunit Aga1p on said yeast cell surface;

labeling said fusion protein by binding a distinctive label to at least one of said second epitope tag and said mutant polypeptide;
employing flow cytometry to sort yeast cells according to their labeling characteristics;
determining the surface expression level of said fusion protein in said sorted cells; and
determining the ligand binding characteristics of said mutant polypeptide.

37. The DNA intermediate product of the process of claim 36 said gene coding for the desired mutant polypeptide product of said process.

38. A kit for producing a yeast cell displayed variant ligand biding protein with enhanced binding properties relative to a wild-type of said ligand binding protein, the kit comprising:

expression cassettes capable of being covalently ligated to individual members of a library of randomly mutated genes encoding a mutated polypeptide, said cassettes each having the structure

5'-GAL 1-10 -- a AGA2p - mutated polypeptide;

a vector capable of accepting said expression cassettes;
said vector useable with yeast cells to yield a multiplicity of transformed yeast cells, capable of expressing said cassettes in said transformed yeast cells, whereby said ligand binding protein is displayed on the surface of each said yeast cell, said displayed ligand binding protein containing one of said mutated binding proteins;
labels for labeling the displayed proteins on said yeast cells by binding a specific label to said displayed proteins, said labels being readable by flow cytometry when used to sort said yeast cells according to their labeling characteristics;
instructions for determining the surface expression level of said ligand binding protein in said sorted cells, and for determining the ligand binding characteristics of said ligand binding protein on the surface of said sorted cells whereby at least one preferred yeast

cell expressing an abundance of ligand binding proteins which exhibits enhanced ligand binding characteristics is identified, and for cloning said at least one preferred yeast cell.

39. A kit for producing a variant T cell binding protein with enhanced T-cell binding properties relative to a wild-type of said T cell binding protein, the kit comprising:
expression cassettes capable of being covalently ligated to a gene encoding said wild-type T cell binding protein, each having the structure

5'-GAL 1-10 -- a AGA2p –HA-- mutated polypeptide –c-myc-3';

a vector capable of accepting said expression cassettes;
said vector useable with yeast cells to yield a multiplicity of transformed yeast cells, capable of expressing said cassettes in said transformed yeast cells, whereby said mutated T cell binding proteins are joined at the N-terminus to the C-terminus of a first epitope tag sequence, the N-terminus of said first epitope tag sequence joined to C-terminus of an agglutinin subunit Aga2p sequence, the T cell binding protein sequence being joined at its C-terminus to a second epitope tag, said Aga2p being joined by at least one disulfide bond to an agglutinin subunit Aga1p on said yeast cell surface;
labels for labeling the displayed proteins on said yeast cells by binding a specific label to said displayed proteins, said labels being readable by flow cytometry when used to sort said yeast cells according to their labeling characteristics, said labels cytometrically distinguishable when used to label c-myc and to said T cell binding protein;
instructions for determining the surface expression level of said T cell binding protein in said sorted cells, and for determining the ligand binding characteristics of said T cell expressing an abundance of T cell binding proteins which exhibits enhanced T cell binding characteristics is identified, and for cloning said at least one preferred yeast cell, and for reducing said at least one disulfide bond whereby said fusion protein is released from said yeast cells.

40. A gene expression cassette comprising in order:

GAL 1-10 promoter

a Aga2p

*

polypeptide

‡

wherein "*" is nothing or a first epitope tag, and

"‡" is nothing or a second epitope tag.

41. The gene expression cassette of claim 40 wherein at least one epitope tag is chosen from group consisting of SEQ ID NO:1; SEQ ID NO:2, SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:9.

42. The gene expression cassette of claim 40 wherein at least one epitope tag is SEQ ID NO:1.

43. The gene expression cassette of claim 40 wherein at least one epitope tag is SEQ ID NO:2.

44. The gene expression cassette of claim 40 wherein the first epitope tag is SEQ ID NO:1 and the second epitope tag is SEQ ID NO:2.

45. The cassette of claim 40 wherein said polypeptide is a mutant polypeptide.

46. The cassette of claim 40 wherein said polypeptide is a ligand binding polypeptide.

47. The cassette of claim 46 wherein said ligand binding polypeptide is an antibody or fragment thereof capable of antigen-specific binding.

48. The cassette of claim 47 wherein said antibody or fragment thereof is an anti-T cell fragment chosen from the group consisting of SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; and SEQ ID NO:24.

49. The cassette of claim 47 wherein said antibody or fragment thereof is a single chain antibody binding site.

50. The cassette of claim 40 wherein said ligand binding polypeptide is a receptor or fragment thereof.

51. The cassette of claim 50 wherein said receptor is a cell surface receptor.

52. The cassette of claim 51 wherein said cell surface receptor or fragment thereof is a T cell receptor or fragment thereof.

53. The cassette of claim 51 wherein said cell surface receptor or fragment thereof is a mutant T cell receptor or fragment thereof.

54. The cassette of claim 53 wherein said T cell receptor or fragment thereof is SEQ ID NO:24.

55. A vector for transforming a yeast cell said vector containing the expression cassette of claim 40.

56. The vector of claim 55 wherein at least one epitope tag is chosen from group consisting of SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:9.

57. The vector of claim 55 wherein at least one epitope tag is SEQ ID NO:1.

58. The vector of claim 55 wherein at least one epitope tag is SEQ ID NO:2.

59. The vector of claim 55 wherein the first epitope tag is SEQ ID NO:1 and the second epitope tag is SEQ ID NO:2.

60. The vector of claim 55 wherein said polypeptide is a mutant polypeptide.

61. The vector of claim 55 wherein said polypeptide is a ligand binding polypeptide.

62. The vector of claim 55 wherein said ligand binding polypeptide is an antibody or fragment thereof capable of antigen-specific binding.

63. The vector of claim 61 wherein said antibody or fragment thereof is an anti-T cell fragment chosen from the group consisting of SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; and SEQ ID NO:24.

64. The vector of claim 62 wherein said antibody or fragment thereof is a single chain antibody binding site.

65. The vector of claim 61 wherein said ligand binding polypeptide is a receptor or fragment thereof.

66. The vector of claim 65 wherein said receptor is a cell surface receptor.

67. The vector of claim 66 wherein said cell surface receptor or fragment thereof is a T cell receptor or fragment thereof.

68. The vector of claim 66 wherein said cell surface receptor or fragment thereof is a mutant T cell receptor or fragment thereof.

69. The vector of claim 68 wherein said T cell receptor or fragment thereof is SEQ ID NO:24.

70. A method for using high affinity TCRs to identify ligands comprising:
labeling high affinity TCRs;
contacting said labeled TCRs with ligands;
identifying the ligand with which the labeled TCR is bound.

71. The method of claim 70, wherein said label is selected from the group consisting of: fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.

72. The method of claim 70, wherein said ligands are peptide/MHC ligands.

73. A method of using high affinity TCRs to bind to a selected peptide/MHC ligand comprising:
labeling said high affinity TCRs with a label that binds to the selected peptide/MHC ligand;
contacting said labeled high affinity TCRs with cells containing MHC molecules.

74. The method of claim 73, wherein said label is selected from the group consisting of: fluorescent compounds, chemiluminescent compounds, radioisotopes and chromophores.

75. The method for blocking autoimmune destruction of cells comprising:
contacting TCRs with high affinity for the site recognized by the T lymphocytes on the surface of a target cell with cells, whereby the autoimmune destruction of cells is blocked.

76. The method for using high affinity TCRs to treat disease comprising:
coupling a TCR having a high affinity for a neoplastic cell surface marker with a therapeutic compound; and
contacting said TCR with cells.

77. Soluble T cell receptors (TCRs) having higher affinity for a ligand than wild type TCRs.

78. The soluble high affinity TCRs of claim 77, wherein said ligand is a peptide/MHC ligand.

79. The soluble high affinity TCRs of claim 77, wherein said high affinity TCR is made by the method comprising: mutagenizing a TCR to create mutant TCR coding sequences; transforming DNA comprising the mutant TCR coding sequences for mutant TCRs into yeast cells; inducing expression of the mutant TCR coding sequences such that the mutant TCRs are displayed on the surface of yeast cells; contacting the yeast cells with a fluorescent label which binds to the peptide/MHC ligand to produce selected yeast cells; and isolating the yeast cells showing the highest fluorescence.

80. The soluble high affinity TCRs of claim 77 isolated by yeast display.

81. DNA library comprising nucleic acids encoding soluble high affinity TCRs, wherein said TCRs are made by the method of mutagenizing a TCR to create mutant TCR coding sequences; transforming DNA comprising the mutant TCR coding sequences for mutant TCRs into yeast cells; inducing expression of the mutant TCR coding sequences such that the mutant TCRs are displayed on the surface of yeast cells; contacting the yeast cells with a fluorescent label which binds to the peptide/MHC ligand to produce selected yeast cells; and isolating the yeast cells showing the highest fluorescence.

82. A library of T cell receptor proteins displayed on the surface of yeast cells which have higher affinity for the peptide/MHC ligand than the wild type T cell receptor protein, wherein said library is formed by mutagenizing a T cell receptor protein coding sequence to generate a variegated population of mutants of the T cell receptor protein coding sequence; transforming the T cell receptor mutant coding sequence into yeast cells; inducing expression of the T cell receptor mutant coding sequence on the surface of yeast cells; and selecting those cells expressing T cell receptor mutants that have higher affinity for the peptide/MHC ligand than the wild type T cell receptor protein.

83. A method for cloning the gene for a high affinity TCR mutant into a system that allows

expression of the mutant on the surface of T cells comprising:
mutating TCRs to create high affinity TCR mutants;
cloning said TCR mutants into a vector;
transfecting the vector into T cells;
expressing the high affinity TCR mutant on the surface of T cells.

84. The method of claim 83, further comprising:
selecting those T cells that are activated by a peptide/MHC ligand more than the wild type.

85. The method of claim 83, wherein the transfected/infected T cells are used for recognition of selected peptide-bearing MHC cells.

86. T cells made by the method of claim 83.

87. A DNA sequence encoding a soluble mutant high affinity TCR exhibit higher affinity for its cognate ligand than wild type TCR.

88. The DNA sequence of claim 87 wherein the cognate ligand is a peptide/MHC ligand.

89. The DNA sequence of claim 87 wherein the cognate ligand is superantigen.

90. The DNA sequence of claim 87, which is made by the method comprising: mutagenizing a TCR to create mutant TCR coding sequences; transforming DNA comprising the mutant TCR coding sequences for mutant TCRs into yeast cells; inducing expression of the mutant TCR coding sequences such that the mutant TCRs are displayed on the surface of yeast cells; contacting the yeast cells with a fluorescent label which binds to the peptide/MHC ligand to produce selected yeast cells; and isolating the yeast cells showing the highest fluorescent and thereafter isolating the mutant TCR coding sequences from the selected yeast cells.

91. A method for selecting proteins for displayability on a yeast cell surface, comprising:
transforming yeast cells with a vector which expresses a protein to be tested fused to a yeast cell wall protein, wherein mutagenesis is used to generate a variegated population of mutants of the protein to be tested;
contacting said yeast cells with a label which binds to proteins that are displayed on the yeast cell surface and does not bind to proteins that are not displayed on the yeast cell surface;
isolating said yeast cells with which said label is bound, wherein the presence of said label bound to a protein to be tested indicates said protein to be tested is displayable on the yeast cell surface;
transforming yeast cells with a vector expressing a protein to be tested fused to a yeast cell wall protein, wherein mutagenesis is used to a generate a variegated population of mutants of the protein to be tested;
labeling said yeast cells with a first label, wherein said first label associates with yeast expressing said protein to be tested and does not associate with yeast which do not express said protein to be tested;
isolating said yeast cells with which said first label is associated; and
analyzing and comparing said properties of said mutant protein expressed by yeast with properties of said wild-type protein, wherein yeast cells exhibiting mutant proteins with enhanced properties over the wild-type protein are selected.

92. A pharmaceutical composition comprising a high affinity TCR in a pharmaceutical carrier.

93. The method of using the pharmaceutical composition of claim 92 comprising administering the composition to a patient.

94. The method of claim 1, wherein said isolating step is performed by flow cytometry.

95. The method of claim 2, wherein said quantitating step is performed by flow cytometry.

96. A method of selecting a mutant protein with enhanced displayability over a wild type protein comprising:
mutagenizing a protein coding sequence to generate a variegated population of mutants of the protein coding sequence;
transforming the mutant coding sequence into yeast cells;
inducing expression of the mutant coding sequence on the surface of yeast cells; and
selecting those cells expressing mutants that have enhanced displayability over the wild type protein.

97. A method of diagnosing a disease in a patient comprising:
removing wild-type T cells from the patient;
transforming the T cells with a vector that expresses a marker for the disease;
returning the transformed T cells to a patient;
detecting the marker for the disease.